binax

Proc. #

INSTITUTION: _____ **DATE:** _____

ADDRESS: _____

DEPARTMENT: _____

PROCEDURE: **BINAX NOW® *LEGIONELLA* URINARY ANTIGEN TEST**

PREPARED BY	DATE ADOPTED	SUPERSEDES PROCEDURE #

REVIEW DATE	REVISION DATE	SIGNATURE

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PRINCIPLE:

The Binax NOW® *Legionella* Urinary Antigen Test is an in vitro rapid immunochromatographic assay for the qualitative detection of *Legionella pneumophila* serogroup 1 antigen (*L. pneumophila* serogroup 1 antigen) in urine specimens from patients with symptoms of pneumonia. It is intended to aid in the presumptive diagnosis of *Legionella* infection

(Legionnaires' Disease) caused by *L. pneumophila* serogroup 1 in conjunction with culture and other methods.

Rabbit anti-*Legionella pneumophila* serogroup 1 antibody, the patient line, is adsorbed onto nitrocellulose membrane. Goat anti-rabbit IgG, the control line, is adsorbed onto the same membrane as a second stripe. Rabbit anti-*Legionella pneumophila* serogroup 1 antibodies are conjugated to visualizing particles that are dried onto an inert fibrous support. The resulting conjugate pad and the striped membrane are combined to construct the test strip. This test strip and a well to hold the swab specimen are mounted on opposite sides of a hinged, book-shaped test device.

To perform the test, a swab is dipped into the urine specimen, removed, and then inserted into the test device. Reagent A is added from a dropper bottle. The device is then closed, bringing the sample into contact with the test strip. *L. pneumophila* serogroup 1 urinary antigen captured by immobilized anti-*L. pneumophila* serogroup 1 antibody reacts to bind conjugated antibody. Immobilized goat anti-rabbit IgG also captures visualizing conjugate, forming the control line. A positive test result is read visually in 15 minutes or less depending on the concentration of antigen present in the urine specimen. A negative result, read in 15 minutes, indicates that *L. pneumophila* serogroup 1 antigen was not detected in the urine sample.

The test is interpreted by the presence or absence of visually detectable pink-to-purple colored lines. A positive result will include the detection of both a patient and a control line, while a negative assay will produce only the control line. Failure of the control line to appear, whether the patient line is present or not, indicates an invalid assay.

SPECIMEN:

Urine specimens should be collected in standard containers, stored at room temperature (59-86°F, 15-30°C) and assayed within 24 hours of collection. Alternatively, specimens may be stored at 2-8°C for up to 14 days or at -10°C to -20°C for longer periods before testing. Long term storage conditions have not been determined. Boric acid may be used as a preservative. Other preservatives have not been evaluated.

When necessary, urine specimens should be shipped in leakproof containers at 2-8°C or frozen.

Allow all specimens to equlibrate to room temperature before testing.

MATERIALS AND EQUIPMENT:

Materials:

Test Devices: A membrane coated with rabbit anti-*Legionella pneumophila* serogroup 1 antigen and goat anti-rabbit IgG is combined with rabbit anti-*Legionella pneumophila* serogroup 1 conjugate in a hinged test device.

Reagent A: Citrate / Phosphate with Tween 20 and Azide.

Swabs: Designed for use in the *Binax NOW™ Legionella Urinary Antigen Test*. **Do not use other swabs.**

Positive Control: Heat inactivated *L. pneumophila* serogroup 1 positive urine dried onto swab.

Negative Control: Heat inactivated normal human urine dried onto swab.

Equipment:

clock, timer, or stopwatch

standard urine collection containers

HANDLE URINE SPECIMENS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.

Preparation:

All materials are supplied ready to use.

Performance Considerations:

None

Storage Requirements:

Store kit at room temperature (59-86°F, 15-30°C). The Binax NOW™ *Legionella* Urinary Antigen Test kit and reagents are stable until the expiration dates marked on their outer packaging and containers.

CALIBRATION:

None required.

QUALITY CONTROL:

Daily Quality Control:

The Binax NOW® *Legionella* Urinary Antigen Test contains built-in control features. The manufacturer's recommendation for daily quality control is to document these controls for each sample run.

Positive Procedural Control

The pink-to-purple line at the "Control" position can be considered an internal positive procedural control. If capillary flow has occurred, this line will always appear.

Negative Procedural Control

The clearing of background color in the result window provides a negative background control. The background color in the window should be light pink to white within 15 minutes and should not interfere with the reading of the test result.

External Positive and Negative Controls:

Good Laboratory Practice recommends the use of positive and negative controls to assure functionality of reagents and proper performance of assay procedure. Positive and negative control swabs that will monitor the entire assay are provided in the kit. Alternatively, known positive and negative urine specimens may be run as controls. To use liquid urine controls, simply process as you would a patient sample.

Positive and negative controls should be tested once for each test kit (12 test or 22 test kit sizes), and as otherwise required by your laboratory's standard quality control procedures.

Do not report patient test results if expected control results are not obtained.

ASSAY PROCEDURE :

Procedure for Patient Samples (and liquid urine controls):

Bring sample to room temperature. Remove device from the pouch **just before use.** Lay device flat and run test as follows:

1. Dip a swab into the urine sample to be tested, completely covering the swab head. If the swab drips, touch swab to side of urine container to remove excess urine.
2. There are two holes on the inner right panel of the device. Insert swab into the **BOTTOM** hole. Firmly push upwards so that the swab tip is fully visible in the top hole. **Do not remove the swab**.
3. Hold Reagent A vial vertically, 1 to 1 ½ inches above the device. Slowly add two (2) drops of Reagent A to the **BOTTOM HOLE**.
4. Peel off adhesive liner from the right edge of the test device. Close and securely seal the device. Read result in window 15 minutes after closing the device. Strongly positive patients may be visible sooner.

NOTE: For convenience, the swab shaft has been scored and may be snapped off **after** closing the device. Avoid dislodging the swab from the well when doing so.

Procedure for Binax *NOW*® Swab Controls:

Remove device from the pouch **just before use.** Lay device flat and run test as follows:

1. There are two holes on the inner right panel of the device. Insert swab into the **BOTTOM** hole. Firmly push upwards so that the swab tip is fully visible in the top hole. **Do not remove swab.**
2. Hold Reagent A vial vertically, 1 to 1 ½ inches above the device. Slowly add six (6) drops of Reagent A to the **BOTTOM HOLE**.
3. Peel off adhesive liner from the right edge of the test device. Close and securely seal the device. Read result in window 15 minutes after closing the device. Positive Control Swab may be visible sooner.

CALCULATIONS:

None required.

INTERPRETATION OF RESULTS:

A **negative sample** will give a single control line in the top half of the window, indicating a presumptive negative result. This control line means that the detection part of the test was done right, but no *L. pneumophila* serogroup 1 antigen was detected.

A **positive sample** will give two lines. This means that *L. pneumophila* serogroup 1 antigen was detected. Specimens with low levels of antigen may give a faint patient line. **Any visible line is positive.**

If no lines are seen, or if just the patient line is seen, the assay is **invalid**. Invalid tests should be repeated.

REPORTING RESULTS:

RESULT	RECOMMENDED REPORT
Positive	Presumptive positive for *L. pneumophila* serogroup 1 antigen in urine, suggesting current or past infeciton.
Negative	Presumptive negative for *L. pneumpohila* serogroup 1 antigen in urine, suggesting no recent or current infection. Infection due to *Legionella* cannot be ruled out since other serogroups and species may cause disease, antigen may not be present in urine in early infection, and the level of antigen present in the urine may be below the detection limit of the test.

PROCEDURE NOTES:

1. To ensure delivery of an adequate volume of Reagent A, hold vial vertically, 1 - 1 1/2 inches above the test device. Add drops slowly.
2. For *in-vitro* Diagnostic Use.

3. Test device must remain sealed in its protective foil pouch until just before use.
4. Do not use kit past the expiration date printed on the kit box.
5. Do not mix components from different kit lots.
6. Swabs in the kit have been validated for use in the *Binax NOW™ Test*. Do not use other swabs.
7. Solutions used to make the control swabs are processed using standard methods of microorganism inactivation. However, patient samples, controls, and test devices should be handled as though they could transmit disease. Observe established precautions against microbial hazards.

LIMITATIONS OF THE PROCEDURE:

The Binax NOW® *Legionella* Urinary Antigen Test has been validated using urine samples only. Other samples (e.g., plasma, serum or other body fluids) that may contain *Legionella* antigen have not been evaluated.

This test will not detect infections caused by other *L. pneumophila* serogroups and by other *Legionella* species. A negative antigen result does not exclude infection with *L. pneumophila* serogroup 1. Culture is recommended for suspected pneumonia to detect causative agents other than *L. pneumophila* serogroup 1 and to recover *L. pneumophila* serogroup 1 when antigen is not detected in urine.

The diagnosis of Legionnaires' Disease cannot be based on clinical or radiological evidence alone. There is no single satisfactory laboratory test for Legionnaires' Disease. Therefore, culture results, serology and antigen detection methods should be used in conjunction with clinical findings to make an accurate diagnosis.

Excretion of Legionella antigen in urine may vary depending on the individual patient. Antigen excretion may begin as early as 3 days after onset of symptoms and persist for up to 1 year afterwards. A positive result can occur due to current or past infection and therefore is not definitive for infection without other supporting evidence.

Performance of the test on diuretic urine has not been evaluated.

The test has been evaluated on hospitalized patients only. An outpatient population has not been evaluated.

REFERENCES:

1. Fraser, D.W., T.R. Tsai, W. Orensein, W.E. Parkin, P.H., H.J. Beecham, R.G. Sharrar, J. Harris, G.F. Mallison, S. M. Martin, J.E. McDade, C.C. Shepard, P.S. Brachman, and The Field Investigation Team. Legionnaires' disease: description of an epidemic of pneumonia. N. Engl. J. Med. 1977;**297**:1189-1197.
2. Marston, B.J., H.B. Lipman, R. F. Breiman. Surveillance for Legionnaires' Disease: risk factors for morbidity and mortality. Arch. Intern. Med. 1994;**154**:2417-2422.

3. Horwitz, M. A., B.J. Marston, C.V. Broome, and R.F. Breiman. Prospects for vaccine development. Presented at the 4th International Symposium on *Legionella*, 1992. In: Barbaree, J. M., R.F. Breiman, and A. P. DuFour, eds. *Legionella*: Current Status and Emerging Perspectives. Washington, D.C. American Society for Microbiology, 1993.
4. Kohler, R.B. Antigen detection for the rapid diagnosis of Mycoplasma and *Legionella* pneumonia. Diagn. Microbiol. Infect. Dis. 1988;**4**:47S-59S.
5. Roig, J., X. Aquiler, J. Ruiz, et. al. Comparative study of *Legionella pneumophila* and other nosocomial-acquired pneumoniaes. 1991;**99**:344-50.
6. Carretala, J., F. Gudiol, R. Pelleres, et. al. Risk factors for *nosocomial Legionella pneumophila* pneumonia. Am. J. Respir. Crit. Med. 1994;**149**:625-9.
7. Reingold, A.L., B.M. Thomason, B.J. Brake, L. Thacker, H.W. Wilkinson, and J.N. Kuritsky. *Legionella* pneumonia in the United States: the distribution of serogroups and species causing human illness. J. Infect. Dis. 1984;**149**:819.
8. Stout, J.E., V.L. Yu. Legionellosis. New Eng. J. of Medicine. 1997;**337**:682-7.
9. Edelstein, P.H. Legionnaires' Disease. Clinical Infectious Diseases. 1993;**16**:741-9.
10. Berdal, B.P., C.E. Farshy, and J.C. Feeley. Detection of *Legionella pneumophila* antigen in urine by enzyme-linked immunospecific assay. J. Clin. Microbiol. 1979;**9**:575-578.
11. Tilton, R.C. Legionnaires' disease antigen detected by enzyme-linked immunosorbent assay. Ann. Intern. Med. 1979;**90**:697-698.
12. Kohler, R.B., S.E. Zimmerman, E. Wilson, S.D. Allen, P.H. Edelstein, L.J. Wheat, and A. White. Rapid radioimmunoassay diagnosis of Legionnaires' Disease. Ann. Intern. Med. 1981;**94**:601-605.
13. Bibb, W.F., P.M. Arnow, L. Thacker, and R.M. McKinney. Detection of soluble *Legionella pneumophila* antigens in serum and urine specimens by enzyme-linked immunosorbent assay with monoclonal and polyclonal antibodies. J. Clin. Microbiol. 1984;**20**:478-482.
14. Tang, P.W., and S. Toma. Broad-spectrum enzyme-linked immunosorbent assay for detection of *Legionella* soluble antigens. J. Clin. Microbiol. 1986;**24**:556-558.
15. Kohler, R.B., W.C. Winn, Jr., and L.J. Wheat. Onset and duration of urinary antigen excretion in Legionnaires' disease. J. Clin. Microbiol. 1984;**20**:605-607.

For Technical Assistance or Ordering, call or write:

Binax, Inc.
217 Read Street
Portland, ME 04103 USA

(800) 323-3199 Toll free in the U.S.
(207) 772-3988 Outside the U.S.
(207) 761-2074 Facsimile

Website: www.binax.com